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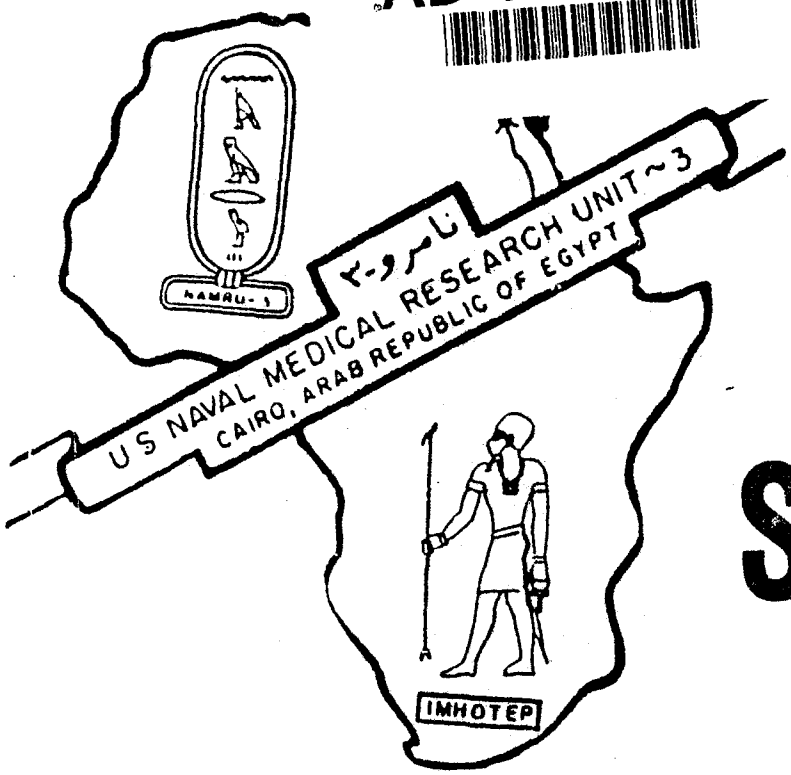

PUBLICATION REPORT

1693

8/92

ISOLATION AND CHARACTERIZATION OF LEISHMANIA MAJOR FROM PHLEBOTOMUS PAPATASI AND MILITARY PERSONNEL IN NORTH SINAI, EGYPT

BY

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Transactions of the Royal Society of Tropical Medicine and Hygiene (1991) 85, 590-591

Short Report

Isolation and characterization of *Leishmania major* from *Phlebotomus papatasi* and military personnel in north Sinai, Egypt*

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Since October 1982, cutaneous leishmaniasis has been repeatedly diagnosed among military members of the multinational force and observers (MFO), an international peace keeping force based in the north Sinai desert of Egypt (DUNN & SMERZ, 1983; MANSOUR *et al.*, 1987). In an effort to assist in the recognition, prevention, and treatment of this disease among MFO personnel, the US Naval Medical Research Unit no. 3 initiated epidemiological studies in July 1989 at locations in north-eastern Sinai.

As part of these epidemiological investigations, an entomological survey sought to determine the seasonal dynamics of local sandflies and the prevalence of *Leishmania* infection among these potential vectors. Sandflies were obtained from human landing and resting collections during the months of July, August and September, 1989. Flies were cryopreserved following a modification of the procedure developed by YOUNG *et al.* (1987). Sandfly pools in cryopreservant were slowly frozen on dry ice and transferred to liquid nitrogen for storage until examined. Cryopreserved sandflies were thawed and individually dissected on autoclaved microscope slides in a drop of sterile phosphate-buffered saline (PBS) containing 200 iu penicillin and 200 µg streptomycin per ml to determine the species, reproductive status, and presence and location of promastigote infections. The intact midgut and surrounding media from those specimens containing promastigotes were removed from the slide, triturated in approximately 1·0 ml of sterile PBS with antibiotics (as above), and inoculated into NNN medium and into the hind footpads of BALB/c mice. Tanabe's medium (TANABE, 1923) was used for initial isolation and maintenance of *Leishmania* obtained by aspiration and biopsy of suspected cutaneous leishmaniasis lesions from patients presenting with typical lesions. Both sandfly and human isolates were subsequently passaged into El On's medium (EL-ON, 1969) and harvested at log growth phase. The promastigotes were centrifuged, washed, resuspended in PBS, and stored at −80°C until isoenzyme electrophoresis was performed. Promastigotes were serotyped according to the method of SCHNUR & ZUCKERMAN (1977), using specific parasite excretory factors (EF) present in the culture medium. Comparative cellulose acetate electrophoresis against World Health Organization reference strains of *L. major* MHOM/IL/67/Jericho-II (=LRC-L137), *L. tropica* MHOM/SU/58/OD (=LRC-L39), and *L. donovani* MHOM/IN/80/DD8 was used to identify 2 sandfly isolates, IPAP/EG/89/Si-177 and IPAP/EG/90/Si-1614, and 3 human isolates, MHOM/EG/89/Si-15, MHOM/EG/89/Si-17 and MHOM/EG/90/Si-18, from MFO personnel, all from the Sinai study area. The electrophoretic methods of KREUTZER & CHRISTENSEN (1980) were used for the separation of glucose-6-phosphate dehydrogenase (G6PD; EC.1.1.1.49), glucose phosphate isomerase (GPI; EC.5.3.1.9), mannose phosphate isomerase (=phosphomannose isomerase: MPI; EC.5.3.1.8), phosphoglucomutase (PGM; EC.2.7.5.1) and 6-phosphogluconic dehydrogenase (6PGD; EC.1.1.1.44), and that of HARRIS & HOPKINSON (1976) for the visualization of malate dehydrogenase (MDH; EC.1.1.1.37).

Phlebotomus papatasi was the only man-biting sandfly species identified in the 1594 specimens dissected. The promastigote infection rate for this sample, representing the period July–September 1989, was approximately 0·7% (11/1594). Promastigotes from 2 sandfly infections were successfully cultured in NNN medium, and produced lesions after inoculation into the footpads of BALB/c mice. Serotyping of both the sandfly isolates revealed that they were of the same serotype as the 2 recent MFO isolates (EF serotype A_1B_2) and also several other human *Leishmania* isolates from the same area in the Sinai (MANSOUR *et al.*, 1987). In addition, each isolate yielded isoenzyme profiles identical to those of the *L. major* reference strain used for comparison (Figure).

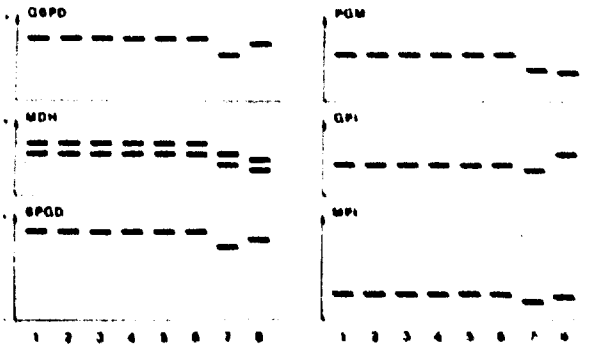

Figure. Diagrammatic representation of 6 isoenzyme electrophoretic patterns on cellulose acetate for *Leishmania* isolated from two *Phlebotomus papatasi* and 3 human cases with cutaneous lesions (acquired in Sinai) compared to World Health Organization (WHO) reference strains. Numbers correspond to isolates and WHO strains, as follows (1) *L. major* MHOM/IL/67/Jericho-II; (2) MHOM/EG/89/Si-15; (3) MHOM/EG/89/Si-17; (4) MHOM/EG/90/Si-18; (5) IPAP/EG/89/Si-177; (6) IPAP/EG/90/Si-1614; (7) *L. tropica* MHOM/SU/58/OD; (8) *L. donovani* MHOM/IN/80/DD8. See text for explanation of abbreviated names of enzymes.

*This work was supported by the US Naval Medical Research and Development Command, Bethesda, Maryland, Work Unit no. 3M162770A870.AR.322. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department, Department of Defense, or the US Government.

Requests for offprints: Research Publications Branch, US Naval Medical Research Unit no. 3 (NAMRU-3), FPO, AE 09835-1600, USA.

It is reasonable to consider the 10 unidentified promastigote infections in the wild-caught sandflies as *L. major*. This consideration is based upon their position in the sandfly midgut and the tendency of *P. papatasi* to feed preferentially upon warm-blooded hosts. Their position in the midgut, and not in the hind-gut, excludes the possibility of their being trypanosomatids of reptiles, upon which *P. papatasi* only occasionally feeds (KILLICK-KENDRICK, 1979). Because *Leishmania* isolated from the naturally-infected sandflies in this study was indistinguishable from the parasite isolated from cutaneous lesions in man, *P. papatasi* is considered to be a grade 3 vector (KILLICK-KENDRICK & WARD, 1981) in Egypt. WAHBA *et al.* (1990) had previously characterized a single isolate of *L. major* from Egyptian collections of *P. papatasi*. However, this is the first report of repeated, concurrent isolations of this parasite from both sandflies and humans in Egypt.

Acknowledgements

We acknowledge, with appreciation, confirmatory isoenzyme electropheresis of human and sandfly isolates by Dr Richard Kreutzer of the Youngstown State University, Youngstown, Ohio, USA. Grateful thanks are extended to members of the multinational force and observers for their participation.

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Received 20 September 1990; revised 7 February 1991; accepted for publication 8 February 1991

Announcement

Preliminary Announcement

The second residential meeting of the Royal Society of Tropical Medicine and Hygiene (to include the Annual General Meeting) and other European Societies of Tropical Medicine will be held at the Royal College of Physicians, Edinburgh, Scotland from Monday 5th to Wednesday 7th July 1993. Further details available shortly from the Administrator, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W1N 4 EY, UK (Tel: 071 580 2127; Fax: 071 436 1389).

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	20 SEP 90	

4. TITLE AND SUBTITLE
Isolation and Characterization of Leishmania major from Phlebotomus papatasi and Military Personnel in North Sinai, Egypt.

5. FUNDING NUMBERS
PE- 62770A
WU- 3M162770A870.AR.322

6. AUTHOR(S)
Mansour, N.S., Fryauff, D.J., Modi, G.B., Mikhail, E.M. and Youssef, F.G.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Naval Medical Research Unit No. 3
PSC 452, Box 5000
FPO AE 09835-0007

8. PERFORMING ORGANIZATION REPORT NUMBER
8/92

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Naval Medical Research and Development
Command, National Naval Medical Center
Building 1, Tower 12
Bethesda, MD 20889-5044

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

Accession For
NTIS GRA&I
DTIC TAB
Unannounced
Justification
By
Distribution/
Availability Codes
Dist | Avail and/or Special
A-1 | 20

11. SUPPLEMENTARY NOTES
Published in: Trans. R. Soc. Trop. Med. Hyg., 85:590-591, 1991; Acc. No. 1693.

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
Distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Cutaneous leishmaniasis (CL) was repeatedly diagnosed among members of the Multinational Forces and Observers (MFO) in the north Sinai. Leishmania parasites were isolated from both sandflies and suspected human cases of (CL). This study revealed that Phlebotomus papatasi is the only man-biting species in this area. Leishmania parasites produced lesions in the footpads of BALB/c mice, their excretory factor (EF) gave the serotype A_1B_2, and each isolate yielded isoenzyme profiles identical to those of the L. major reference strain. This is the first report of repeated, concurrent isolation of L. major from both sandflies and humans in Egypt.

14. SUBJECT TERMS
Cutaneous leishmaniasis; Diagnosis; Leishmania major; Phlebotomus papatasi; MFO personnel; North Sinai, Egypt.

15. NUMBER OF PAGES
2

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	